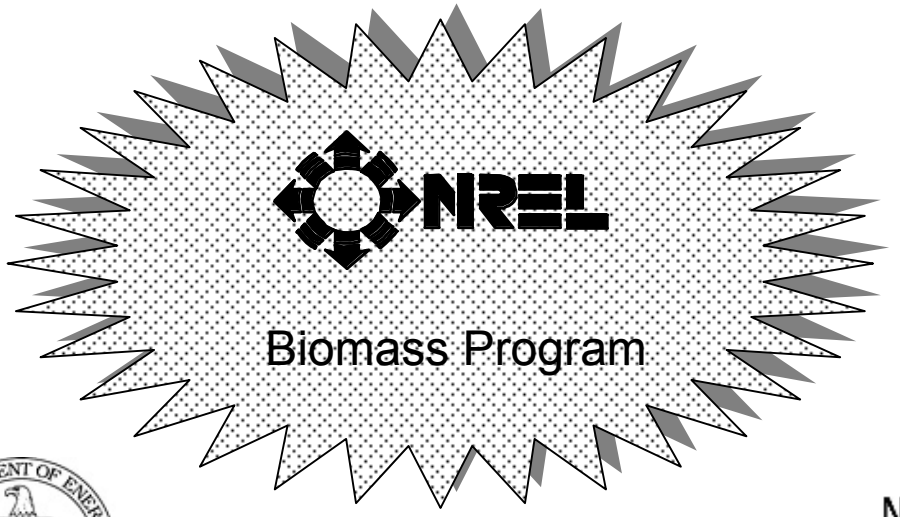

Biomass Analysis Technology Team
Laboratory Analytical Procedure

DRAFT
Version 2004

Procedure Title:
Determination of Protein Content in Biomass

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1. Introduction

1.1 Many types of biomass are used as a feedstock for conversion to fuels and chemicals contain protein and other nitrogen containing materials. These constituents must be measured as part of a comprehensive biomass analysis. Protein in biomass is not always directly measured. In many cases the nitrogen content of the biomass sample is measured and the protein content is estimated using an appropriate Nitrogen Factor (NF). Many standard methods recommend use of an NF of 6.25 for all types of biomass except wheat grains where an NF of 5.70 is recommended. This LAP includes a procedure for calculating an appropriate NF for any biomass type.

1.2 The procedure for the determination of Nitrogen Factors is substantially similar to that described in Mossé (1990)

1.3 This procedure covers the determination of nitrogen-to-protein conversion factors that are used to estimate the amount of protein in a biomass sample.

2. Scope

2.1 This procedure is appropriate for most types of biomass, including extractives-free biomass (biomass that has been extracted using LAP "Determination of Extractives in Biomass") and biomass before extraction.

2.2 This procedure is suitable for process solids but it has not been validated for measuring protein in process liquids.

2.3 Many types of biomass feedstocks, such as bark-free wood chips, contain little or no protein. For these samples, a protein measurement is not a necessary part of a comprehensive compositional analysis.

2.4 All analyses shall be performed according to the guidelines established in an appropriate laboratory specific Quality Assurance Plan (QAP

2.5 Results are reported as a weight percent of the oven-dried biomass (105ºC).

3. Terminology

3.1 *Oven dry weight (ODW)*- the weight of biomass mathematically corrected for the amount of moisture present in the sample at the time of weighing

3.2 *Nitrogen Factor* – A conversion factor used to estimate protein content from a measured nitrogen content.

3.3 Non-protein nitrogen – The difference between the measured nitrogen content and the nitrogen content that can be accounted for in protein. This includes, but is not limited to, nitrogen found in nitrates, nitrites, chlorophyll, nucleic acids an free amino acids.

4. Significance and Use

4.1 This procedure is used, possibly in conjunction with other procedures, to determine the amount of protein and other nitrogen containing materials in a solid biomass sample.

4.2 This procedure is used at several stages in a comprehensive compositional analysis of a biomass sample, See LAP "Summative Mass Closure for Biomass Samples".

5. Interferences

5.1 In this procedure the protein content of a biomass sample is estimated using the results of other determinations (nitrogen content and amino acid profile). The precision and accuracy of those methods are reflected in the results.

5.2 Biomass samples containing less than 0.2 weight percent protein may fall below the validated range of analysis for some standard methods. AOAC 990.03 for crude protein by combustion states that the methods is applicable to solids samples containing 0.2% – 20% nitrogen

5.3 Proteins in biomass may be structurally modified during pretreatment and saccharification. Standard amino acid determinations may not quantify these portions of the protein. Assumption made in this method concerning the relationship between nitrogen content and protein content may no longer be valid.

6. Apparatus

6.1 none

7. Reagents and materials

7.1 Reagents

7.1.1 none

7.2 Materials

7.2.1 This procedure require a measurement of sample nitrogen content. This measurement can be a combustion measurement performed according to AOAC 990.03 or a Kjeldahl measurement performed according to AOAC 984.13.

7.2.2 This procedure require a complete amino acid profile performed according to AOAC 982.30 E(a,b,c), CHP 45.3.05 (1995) or an equivalent method.

7.2.3 Parts of this procedure require Nitrogen Factor Calculator worksheet, "NF calculator.xls" included as an appendix to this method.

8. ES&H Considerations and Hazards

8.1 Follow all applicable NREL chemical handling procedures

9. Sampling, Test Specimens and Test Units

9.1 Many CHN analyzers have a sample size limit of 100 mg or less. Further size reduction may be necessary to assure that sample being analyzed is representative of the larger biomass sample.

10. Procedure

10.1 Calculating an appropriate Nitrogen Factor.

10.1.1 Obtain a complete amino acid profile of each biomass sample performed according to AOAC 982.30 E(a,b,c), CHP 45.3.05 (1995). If necessary, convert all values to a percent dry weight basis.

10.2 Obtain a measurement of the nitrogen content of each biomass sample. If necessary, convert the values to a percent dry weight basis.
10.3 Enter the amino acid and nitrogen weight percent values into the Nitrogen calculator spreadsheet.
10.4 Calculate k_A, k_P, k_1, k_2 and avg(k_1, k_2) according to the equations shown in section 11
10.5 The average of k_A and k_P is a customized NF for this sample.

11. Calculations

11.1 The N-factor limits in the NF calculator are calculated from amino acid and nitrogen data.
11.2 The upper limit is defined as:

$$k_A = \sum E_i / \sum D_i$$

Where:
E_i = the grams of the *i*th Amino Acid per100 grams of sample (dry weight basis)
D_i = the grams nitrogen of the *i*th Amino Acid per100 grams of sample (dry weight basis)

11.3 The lower limit is defined as:

$$k_P = \sum E_i / N$$

Where:
E_i = the grams of the *i*th Amino Acid per100 grams of sample (dry weight basis)
N = the grams nitrogen per100 grams of dry sample

11.4 A range of highest probability (k_1, k_2) is calculated as follows:

$$k_1 = avg(k_A, k_P) + 0.25(avg(k_A, k_P))$$

and

$$k_2 = avg(k_A, k_P) - 0.25(avg(k_A, k_P))$$

11.5 The appropriate N-Factor is avg(k_A, k_P).

11.6 The protein content of this sample is calculated as follows:

$$\%protein = \%nitrogen \times NF$$

Where:
NF = nitrogen factor = N-Factor

12. Report Format

12.1 Required section

13. Precision and Bias

13.1 Report protein as a weight percent of the oven-dried biomass. Report average and RPD.

14. Quality Control

14.1 Reported Significant Figures or decimal places: Determined by data quality objectives and laboratory specific Quality Assurance Plan, see LAP "Rounding and Significant Figures".

14.1.1 Replicates: Analyses should be performed in duplicate wherever possible

14.1.2 Relative percent difference criteria: Determined by data quality objectives and laboratory specific Quality Assurance Plan

14.2 Blank: not applicable

14.3 Relative percent difference criteria: not applicable

14.4 Calibration verification standard: not applicable

14.5 Sample size: 0.5 grams

14.6 Sample storage: not applicable

14.7 Standard storage: not applicable

14.8 Standard preparation: not applicable

14.9 Definition of a batch: not applicable

14.10 Control charts: not applicable

15. Appendices

15.1 Excel spreadsheet N-Factor calculator.xls

16. References

16.1 Required. See examples below

16.2 Mossé, J., "Nitrogen to Protein Conversion Factor for 10 Cereals and 6 Legumes or oilseeds – a Reappraisal of Its Definition and Determination – Variation According to Species and to Seed Protein Content", 1990, Journal of Agriculture and Food Chemistry Vol. 38(1),18-24.

16.3 Jones, D. "Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins." U.S. Department of Agriculture, 1931, Circular(183): 21

16.4 Mosse, J., J. C. Huet, et al. "The Amino-Acid Composition of Wheat-Grain as a Function of Nitrogen-Content." Journal of Cereal Science, 1985, 3(2): 115-130.

16.5 Tkachuk, R. "Amino acid composition of wheat flours." Cereal Chemistry, 1966, 43: 207-223.

16.6 Tkachuk, R. (). "Nitrogen-to-protein conversion factors for cereals and oilseed meals." Cereal Chemistry, 1969, 46: 419-423.